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LABORATORY PROCEDURE IN WILDLIFE FOOD STUDIES<sup>1/</sup>

Prepared in the Division of Wildlife Research

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INTRODUCTION

This manual, based on experience of research workers of the Fish and Wildlife Service (formerly the Bureau of Biological Survey) over more than half a century, is issued for the guidance of investigators studying the food and feeding habits of vertebrate wild animals.

<sup>1/</sup> This leaflet supersedes Leaflet Bi-1515, Outline of Procedure for Recording Data Obtained in Stomach Examinations, and supplements Leaflet BS-30, Economic Ornithology and the Correlation of Laboratory and Field Methods. For suggestions on procedure in collecting, preserving, and submitting specimens, see Wildlife Leaflet 193, Directions for Collecting Materials for Food Habits Studies.

Though each study is in some degree unique, requiring a certain measure of individuality in approach, nevertheless, conformity within limits of practicability to certain standardized procedures is desirable, as data obtained by different investigators should be comparable. Only thus can data from diverse sources have easy and widespread comprehension and detached fragments of information be available for assembly into complete, useful reports.

New and better methods should be constantly sought, but innovations in procedure should be adopted only after a careful weighing of their effect on all objectives--secondary as well as primary. In general it should be emphasized that in food studies the quality of the output is more important than the quantity.

The manual deals with matters directly related to wildlife food analyses and the recording of data, without discussing filing systems, reference collections, or storage of unexamined material.

As food investigations are chiefly concerned with analyses of stomachs and crops, it appears desirable to present under the heading Stomachs and Crops all procedures that may be involved in these studies. Thus, those general procedures that are likely to be used in any and all food analyses, such as Segregation of Materials, Measurement, Enumeration, Identification, and Re-examination, are included here with special topics--Preliminary Washing, Large Stomachs, Crops, and Gullets--that pertain particularly to stomachs and crops. Consequently, there will be discussed under Droppings, Pellets, and Other Materials such remaining matters as are concerned only with these studies.

## STOMACHS AND CROPS

### Preliminary Washing

Contents of stomachs and crops are generally washed in water preliminary to examination. This is particularly desirable when formaldehyde has been used as a preservative, as this chemical is irritating to membranes of the eyes and nose as well as to the hands. Ordinarily the stomach or crop contents are rinsed in flowing water in shallow, white-enamel pans; gravel and other heavy objects sink to the bottom, but light, floating material can be decanted onto a funnel over which fine-mesh silk bolting cloth has been stretched. The bolting cloth, used as a sieve, should first be wet with alcohol to facilitate rapid passage of water; otherwise, surface tension or clogging by grease is likely to occur. Any residue left on the cloth is removed with a scalpel and placed on small squares of blotting paper. A partial segregation can be effected in conjunction with washing if each decantation of material is kept separate.



The stomach contents of a predatory mammal usually require thorough washing with hot water, applied with pressure, in order to reduce or eliminate grease. For this work a coarse-meshed sieve is essential. The washed contents are squeezed with the hand to remove excess water and placed in a pasteboard box, which is in turn put in a drying oven. Occasionally it is readily evident that fatty materials dissolved by the hot-water treatment constitute a considerable proportion of the stomach contents. In such cases a note as to the proportion thus lost and the identity of the material should be placed in the box, together with the residue of contents, to aid the examiner in calculating percentages.

Not infrequently, when--as in quail or doves--the food is mainly seeds, washing is omitted, and the stomach or crop content is examined "as is." This procedure, when feasible, saves considerable time. On opening the stomach, the character of its contents should decide whether it is to be examined dry, moist, or in a water bath. Sometimes certain items, such as soft-bodied insects, spiders, or crustaceans, should be kept wet until identified and the rest may be dried.

#### Segregation of Materials

Segregation may be accomplished by various means, depending on the kinds of materials being sorted and, to some extent, on the equipment available. Slow and meticulous separation by forceps is often necessary. Leaves and other objects that can be recognized and sorted better when wet cannot be handled so rapidly as dry materials. Among the more elementary means of segregation are floating and decanting, mentioned previously, as well as separation of dry objects of various sizes and shapes by shaking, rolling, or sliding on sheets of paper. Sieves are particularly useful, and a very practical arrangement consists of a series of interfitting cuplike metal sieves (geologists' sieves) through which is forced a blast of air with pressures ranging from about 5 to 50 pounds. This method makes possible almost instantaneous separation of several different sizes of seeds or other objects.

Another mechanical arrangement that has proved helpful in preliminary segregation is the Porter seed blower, used at experiment stations and elsewhere for seed analysis. This device, functioning on principle of gravity differential between items and on readily gaged air pressures applied in a special metal tube, is particularly useful in isolating grit from other objects and in sorting materials of different sizes or densities. In food studies it has been found advantageous to modify the Porter instrument by introducing greater air pressure and by substituting a glass tube in which the progress of separation can be observed.

In many cases, segregations can and should be reasonably complete, but in others this is impracticable.

## Measurement

Measurement of food quantities and of proportions of one kind of food to others, is accomplished in either of two ways, depending on the nature of the materials encountered. Volumetric measurement is usually undertaken in the case of items that are readily segregated and easily measured, but visual estimates of proportions are made as a basis for percentage calculations when the objects eaten are either too large or too irregular to fit into glass graduates or are so small and numerous as to make segregation impracticable.

Volume can be measured by use of glass graduates suited to the purpose. In addition to the medium-sized graduates (5 cc or more) available commercially, it is desirable to have several smaller ones ordinarily "home-made" to facilitate measurement of small quantities. Irregular objects, between which considerable air space may be present, can be more accurately measured by using lead shot of various sizes or by immersion in liquid to ascertain displacement.

Visual estimates of relative proportions of quantity are often the only practical procedure. Estimating is particularly likely to be the procedure used in studying foods of herbivores, large predators, and fish-eating birds. An experienced laboratory technician realizes that frequent checking and measuring improve the degree of accuracy of the estimate. Segregation of respective items into distinct piles, or analyses of representative samples of fragmented and mixed materials, will often assist in arriving at suitable estimates of proportions. In some instances it may be impracticable and undesirable to attempt any measurement of either volume or proportionate quantity.

On occasion there should be calculation of the dimensions of certain food items; for example, in the study of fish-eating birds the length or approximate weight of fish consumed may be valuable data.

Obviously, consistency in method of measurement is important within the limits of a single study.

## Enumeration

Precise counting of items is desirable when practicable. Generally, counting can be done easily and quickly if numbers do not exceed 100. For larger numbers estimates are desirable, and evidence of their approximate nature may be given by round numbers (as 200, 500, 1,000). Mechanical aids are available for estimates of numbers, but a simple and efficient expedient consists of partitioning the materials into approximately equal divisions and subdivisions until a basic unit, small enough to be counted readily, is obtained. If a large quantity of small seeds measures 7 cc the approximate total number may be estimated from exact counts of several small quantities, such as  $1/5$  or  $1/10$  cc.

### Identification

Accuracy is vital in all food analyses and is particularly important in identification. Though specific determinations are often important in wildlife management, it is better to be correct as to genus or family than to make unjustified attempts at naming species. Abundant specific determinations do not necessarily reflect credit on the individual investigator; on the contrary, they may indicate a disregard for scientific accuracy.

Specimens referred to specialists for identification should be adequately segregated, suitably labeled, and submitted in containers that will protect against both contamination and loss.

### Re-examination

Occasional review of the examinations of analysts--particularly those of novices--has been found advantageous. In doing this, examined materials are taken at random and re-examined by experienced analysts. The practice not only tends to give those who might need it the additional incentive to accuracy and alertness, but it also helps correct certain practices and errors that might otherwise continue unnoticed.

### Large Stomachs

In the case of ruminants and other animals having large stomachs, it has been found practical to limit analyses to fractional samples of the total contents. In these instances, estimates of proportions are made rather than volumetric measurements. Generally, it is advantageous to scan the whole sample for large recognizable items and, in addition, to make detailed analysis of small representative portions of it. Washing and passing through sieves will help in the separation and identification of items. Since color aids in differentiating materials in the stomach contents of a ruminant, there is advantage in examining the contents in a fresh (unpreserved) state. This can often be done in cold weather if the specimen is frozen in the field and shipped to the laboratory in that condition.

### Crops

It has been found desirable to concentrate on examination of crops in species having this organ well developed (as in gallinaceous and certain other birds). Food materials in crops are little if at all altered from the original proportions consumed, whereas the contents of gizzards almost invariably present changes in proportions as well as transformations in appearance and condition. Crop contents can be analyzed more rapidly and accurately than those of



gizzards, but, of even greater importance, they yield a more correct indication of food preferences. In cases wherein crops are available, gizzards may also be examined to a limited extent to furnish supplementary information on rate of digestion, use of grit, and frequency of occurrence of items.

### Gullets

Gullet materials, often present in fish-eating birds, ducks, and raptors, are frequently as valuable for study as stomach contents and are handled in the same way, since whatever is found in the gullet merely represents material that has not yet reached the stomach.

## OTHER MATERIALS

### Droppings

Droppings that have been collected with systematic planning and care may supply important data for seasons and places inadequately represented by stomachs or crops and also give valuable information on daily activities. This is particularly the case in certain game birds and predatory mammals.

Dry droppings from birds can be pulverized for examination by crushing in a mortar. Dry examination is commonly preferred because it is easier and more rapid, but insect materials treated this way are not so suitable for study as when the droppings have been soaked in water for some time. When very many bird droppings have been gathered in a limited time from a given place, it is often advantageous to combine a number of them (as many as ten or more) into a single mixture. Such a combination may be regarded as a single study unit--small samplings may then be analyzed to represent the whole, but frequently it is advisable to analyze single droppings.

Mammal droppings may be separated for examination by several methods. The simplest is to break them up dry by hand. If very hard, they can be soaked in water containing a small quantity of alcohol to prevent bacterial action. A third method is to wash the droppings through a series of graduated sieves.

Ordinarily, the results from dropping studies are expressed in terms of occurrence (frequency of occurrences or occurrence percentages). Segregation of component items and volumetric measurements are obviously impracticable, partly because of the inherent difficulty in separating materials in droppings and also because quantitative data in such studies are of limited value.

### Pellets

Pellets are composed of disgorged bones, hair, feathers, fruit pits, seeds, and other digestion-resistant materials pressed into pellet shape by the stomach muscles. As they contain practically



all that is indigestible, pellets are useful for determining the occurrence of a food; they are not so practical for estimating volumetric data because the resistant portions may comprise a small or a large part of the original food items, depending on their character.

Pellets may be examined either wet or dry. Dry examinations is the usual procedure, but the wet method is useful for fresh pellets, which are often heavily cemented with mucus. In the latter case the pellets are allowed to soak an hour or two in a tray of water containing some alcohol until softened sufficiently to be easily separated. When examined dry, the pellets are simply pulled apart and the diagnostic structures separated from other material for identification and counting. Particularly resistant parts, as the heads of beetles or the lower jaws of mice, are used for estimating the number of individuals eaten. Volumetric percentages (based on estimates) are commonly used in pellet analyses, partly for the reason that pellets are largely comparable with the stomach contents of the birds studied.

#### Intestinal Material

Some animals, including snakes, have so poorly defined stomachs that the contents of intestine and stomach are best examined together. In general, however, food remains accumulated in the large intestine are most logically treated as are droppings; they are useful in supplying information on kinds of food taken but should not be used for volumetric or percentage computations (other than occurrence).

#### Cheek-pouch Material

Food collected from the cheek pouches of ground squirrels, chipmunks, pocket mice, and other rodents can be examined in much the same manner as crop or stomach contents.

#### Nest and Den Debris

The waste food and food remains accumulating around raptor nests and at the dens of some carnivores, as foxes, may be collected and analyzed as to kinds of food items. If many nests or dens are represented in the debris, percentage of occurrence can be recorded.

### RECORDS

#### Work Sheets

Work sheets, provided for preliminary listing of data, facilitate the preparation of orderly and accurate card reports and also serve as useful accessory records. Though not intended for permanent

filing, work sheets may serve as temporary or permanent private records for the investigator and may be of especial value in preparing monthly reports. Several types of work sheets have been prepared to meet various needs; a facsimile of one is presented in figure 1.

### Examination Cards

Record cards are of two principal types: one is used for stomach or crop examinations (fig. 2), the other for dropping or pellet studies (fig. 3). In addition, in special envelopes (fig. 4), used as a substitute for cards, lengthy analyses of pellets or miscellaneous materials are filed.

Legibility.--Analysis records on cards should be legibly written in carbon ink or typed with black ribbon. In all cases, the examiner's name should be signed in longhand (in carbon ink). Photostat copies are frequently made of the original cards and furnished to co-operators or others; for this and other reasons, the cards should be neat and legible.

Order.--Methodical order and uniformity should be observed in entering items in any given study. In general, the items should be listed systematically--alphabetically or in order of volume, or in some logical combination of both. Animal and plant items should be grouped separately, and it is good practice to place first the one that represents the major or most important food of the species concerned. In special investigations into the extent of predation by fish-eating birds on game fish, foxes on quail, coyotes on deer, badgers on sage grouse, and armadillos on quail (eggs), the items of predation should be so recorded (or underlined in color) as to catch the eye at once and make a complete reading of the card unnecessary.

If the items and data can be tabulated, it will greatly enhance the general utility of the cards and in particular will facilitate indexing and tabulating. In cards having tabular arrangement, indexing will be aided by organizing occurrence data (numbers of seeds, insects, and other materials) in columns near the left margin of the card and volumetric data (cc. and percent) on the right side (fig. 1, 2, and 3). It is desirable for relatively inexperienced investigators to study recent cards of experienced workers and also to consult, for suggestions, the person in charge of files.

Nonfood items.--Grit, gravel, lead shot, trap debris, internal parasites, and other nonfood materials should be classed as such and should be listed separately from bona fide food items. Their volume is recorded as a percentage of the total stomach content, while the volumes and percentages of food should be based on food contents only. Though "pigeonmilk" is food, it is of special nature and source and is recorded apart from the regular food items.

Numerical data.--Percentages--either of volume or of frequency of occurrence--are ordinarily the principal numerical data to be recorded. In recent years, however, in studies on crops or gizzards

of upland game birds, it has been found desirable to record also the basic volumetric figures (in cubic centimeters), since they may have importance in at least two ways: The volumetric data, translated into weight equivalents, have potential value in nutrition investigations; and the cc. figures are regarded as having much advantage over percentages for purposes of statistical compilation.

Miscellaneous suggestions.--When both crop and stomach are examined, the itemized data for each may be recorded separately on the same card.

Determinations accompanied by a question mark often are of value, yet they frequently present unanswerable problems in final tabulations, and hence should be used with discretion. In cases of questioned specific determination it is desirable to accompany the queried entry with a positive one on its more inclusive classification (as genus, family, or order).

When all or a part of the contents is received, rather than the stomach or crop itself (as is frequently the case in ruminant studies), the fact should be noted on the card.

To use the back of the card, turn it over from bottom to top, and enter the accession number in the upper right-hand corner.

When contents are atypical or when stomachs or crops are so nearly empty as to present an untrue picture of food preferences, it is customary for the examiner to write "Do Not Tabulate" prominently on the face of the card.

Verification of data.--The data on the cards should be proof-read with the original collection schedules. Any correction found necessary should be made also in the accession catalog and on the label accompanying the examined material. The spelling of names of items on cards should be verified by the analyst; also the percentages should be checked to see that the totals for animal and plant items agree with those given at the top of the card and that all food percentages combined add to 100.

Revision.--Corrections or other changes on indexed cards should be made in red ink. When changes are made on cards that have already been indexed, it will be necessary to make corresponding changes on the two index cards covering each item concerned. These corrected cards should, therefore, be turned over to the indexer and not be put back in the file until they have been re-indexed. If tabulation sheets have already been prepared, the changes likewise should be made on them.

Record of material held out.--If any food material is held out for identification or for the reference collections, this fact and the quantity withheld should be noted on the card. The accession



number of the stomach should be written plainly on the label of each vial containing any item that is removed. If material is to be sent to a specialist, the essential data concerning the specimen should be included in the vials. If more than one item is involved, symbols (preferably letters or numbers) may be used on cards and vials to represent the items. When material is returned properly identified, suitable entries should be made on the card. The material should then be added to the remainder of the contents and returned to storage. To facilitate this, the examined materials should be kept by the examiner (except when a long delay is expected) until the withheld items are returned.

Record of empty stomachs or crops.--If stomachs or crops are empty, the fact should be noted on the schedules and in the accession catalog, with the date and the examiner's initials. Similar information should be turned in also with reports on special examinations, the cards being marked "Empty."

### Monthly Reports

Each examiner should keep a record of the materials examined and should report each month, on special forms provided for the purpose, the number of units on each species examined. In the case of special cooperative investigations, the name of the person and organization for whom the examination was made should be noted. This information forms the basis for the annual report on examinations completed in the laboratories.

### STORAGE OF EXAMINED MATERIAL

Examined material, or portions thereof, should be stored for any future checking or re-examination that may be desirable.

The material should be thoroughly dried and placed in glass or cardboard vials, together with the original identification tag or other label. The vials should then be stoppered with cotton. If material is stored in a damp condition, alcohol should be added. The contents of crop and gizzard of the same bird should be placed in the same container but should be effectively separated either by a wad of cotton or a piece of cardboard or, preferably, by putting the lesser quantity in a small inner container. Use the smallest vial possible. Do not waste space. The use of cardboard vials should be avoided in damp climates and when stomach contents are greasy or oily. In storing stomach contents large in volume, as those of fish-eating birds, ruminants, and the larger predators, diagnostic samples only need be saved; but a note as to the proportion of the material saved should be entered on the examination card. Essential data, including the scientific name, the permanent accession number, the date of



examination, and the name of the examiner, should be written on the storage vials.

All vials containing examined material should be assembled at the end of each month, sorted by species, and bundled into convenient packets bound and tied with strong cord; each packet should be labeled to show the species, the inclusive accession numbers (only the lowest and highest need be listed, even though the series is not consecutive and complete), the examiner's name, and the month and year in which examined. The material should then be turned over to the accession clerk for permanent storage. Data recorded on these packets are essential for the card index to examined materials and are important in locating particular lots for partial or complete re-examination.









*Colinus virginianus virginianus* Sex -- No. 224217  
 State Alabama Co. Chambers Loc. Lafayette  
 Date 2-8-39 Hr. -- Collector Roy Smith Coll. No. Q1968  
 Stom. -- cc. Crop 4.77 cc. Anim. 4 % Veg. 96 % Grit .03 cc. 1 %  
 Examined by John N. Doe Date June, 1941

Seeds:	cc.	Percent
1 <i>Chamaecrista nictitans</i>	.01	trace
2 <i>Galactia</i> sp.	.03	1
1250 <i>Lespedeza striata</i>	4.40	93
1 <i>Croton glandulosus</i>	.02	trace
1 <i>Cuscuta</i> sp.	trace	trace
Few leaves of <i>Lespedeza</i> sp.	.10	2
Animal food:		
1 <i>Chrysomelidae</i>	.03	1
1 <i>Phalaenidae</i> larva	.15	3
1 <i>Araneida</i>	trace	trace
1 <i>Gastropoda</i> , fragmented	trace	trace

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*Odocoileus virginianus* Sex ♀ No. M37056  
 State Montana Co. Lake Loc. Flathead Forest  
 Date 2-2-36 Hr. - Collector Chas. Shaw & Joe Daur Coll. No. 1  
 Contents  
 Stom. 2.14 cc. Crop -- cc. Anim. 0 % Veg. 100 % Grit -- cc. -- %  
 Examined by John N. Doe Date Jan. 1940

<i>Pseudotsuga taxifolia</i> , needles & twigs	81	%
<i>Pinus</i> (? <i>panderosa</i> )	13	%
<i>Abies</i> sp.	4	%
<i>Juniperus</i> (? <i>scapularum</i> )	2	%
<i>Pinus contorta</i>	trace	

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FIGURE 2. SAMPLE EXAMINATION CARDS FOR STOMACHS AND CROPS;  
A, WITHOUT LEADERS (FOR TYPING); B, WITH LEADERS



Name	Meleagris gallopavo silvestris		Number	N 2158
Type	Feces (5)		Lot No.	--
Locality	Drury Refuge, Taney County, Missouri		Date	March 21, 1941
Collector	D. L. Spencer		Collector's No.	733-737
Gramineae leaves	.6	Euschistus sp.	trace	
Quercus sp.	.2	Hymenoptera	trace	
Zea mays	.2			
Sorghum halepense	trace			
Cornus sp.	trace			
Juniperus sp.	trace			
Examination made by	John N. Doe		Date	July, 1941

FIGURE 3. SAMPLE CARD FOR RECORD OF DROPPING OR PELLET ANALYSES

Name	Number
Type	Lot No.
Locality	Date
Collector	Collector's No.
Condition	Remarks
Examination made by	Date

FIGURE 4. ENVELOPE USED FOR LENGTHY ANALYSES OF PELLETS OR MISCELLANEOUS MATERIALS

